MFP488 Protein Labeling Kit

Order #
MFP-A1235

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

1. Introduction

SUMMARY

Unit size:
3 labeling reactions, 1 reaction vial contains the amount of dye to label 1 mg IgG (MW ~145,000)

Abs./Em.max.: 501/523 nm

MoBiTec's MFP488 Protein Labeling Kit provides a convenient tool to label proteins with our superior MFP488 dye which indicates the following properties:

- brightness
- high emission efficiency
- high fluorescence intensity - high water solubility
- instrument compatibility

MFP488 is spectrally similar to fluorescein and thus compatible with the same excitation sources and filter sets as used for fluorescein. Its protein conjugates are much brighter and more photostable than fluorescein conjugates.

MFP488 labeled proteins have absorption and fluorescence emission maxima of approximately 501 nm and 523 nm, respectively. The MFP488 Protein Labeling Kit contains everything that is needed to perform 3 separate labeling reactions and to purify the resulting labeled protein conjugates. The MFP488 reactive dye has a succinimidyl ester moiety (NHS-ester) that reacts efficiently with primary amines of proteins to form stable dye-protein conjugates.

2. Materials

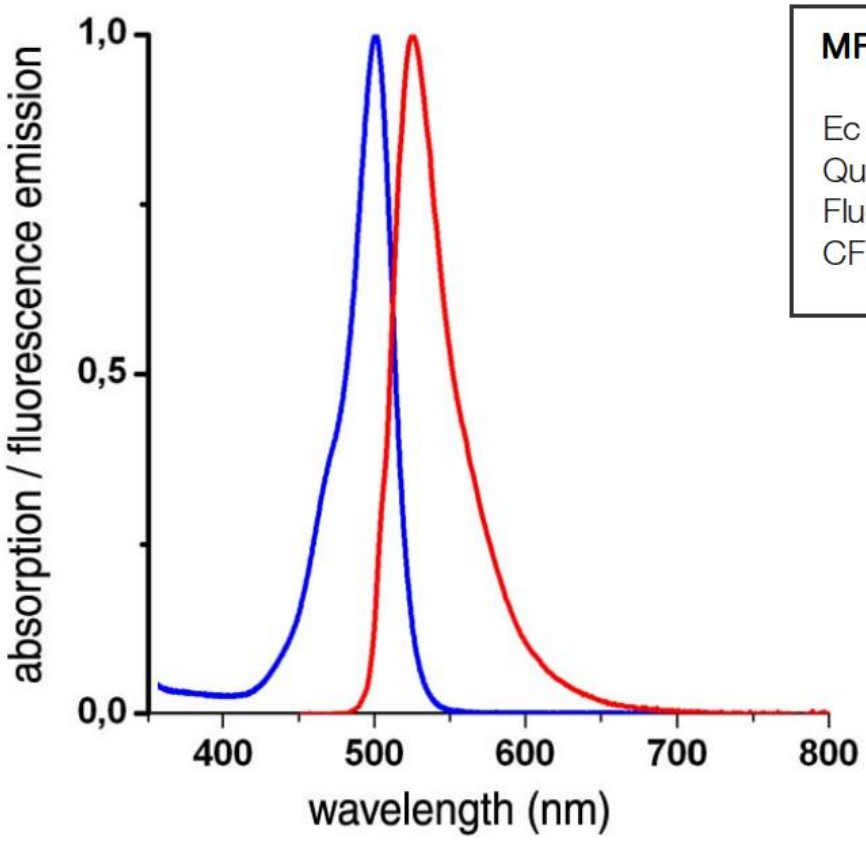
Component A: Reactive dye MFP488 succinimidyl ester (NHS-ester), 3 vials.

Component B: Coupling buffer (1 M Na_2CO_3/$NaHCO_3$, pH 9.0), 10 x concentrated, 500 µl

Component C: Elution buffer (10 x PBS, 20 mM sodium azid, pH 7.4), 10 x concentrated, 10 ml, needs to be diluted to 1 x Elution Buffer

- 3 Purification columns: ready-to-use
- 3 Ultrafiltration cartridges

3. Spectra MFP488 in Water



MFP488 Properties

Ec = 105,000 M^{-1} cm^{-1}
Quantum yield = 82%
Fluorescence lifetime = 3.2 ns
CF (A_{280}/Amax) = 0.09

4. Protein Preparation

Please note: Buffers containing primary amino groups such as Tris or Glycine will interfere with the labeling reaction. The presence of low concentrations of biocides, e.g. sodium azide or thimerosal do not affect the protein labeling reaction.

For optimal labeling efficiency, the purified protein must be in a buffer free of ammonium ions or primary amines. If the protein is in an unsuitable buffer, the buffer should be replaced with phosphate-buffered saline (PBS) by ultrafiltration, please refer to step 8. Impure proteins (e.g. antibodies in crude serum) will not label well.

Each vial of reactive dye contains the appropriate amount of dye to label approximately 1 mg of IgG (MW ~145,000) as 0.2 ml of IgG solution at 5 mg/ml.

5. Protocol

5.1 Labeling Reaction

This kit can be used to label virtually any protein, although the following protocol has been optimized for labeling IgG antibodies. Other antibodies and proteins may also be readily labeled, separation media and techniques may vary in order to produce optimal results.
One reaction vial of the kit is designed to label 1 mg protein. Altering the protein concentration and pH will change the labeling efficiency of the reaction. Optimal labeling generally occurs at pH 9.0.

5.1.1 For labeling reaction we recommend a protein concentration of 2 - 10 mg/ml, for optimal antibody labeling results use 5 mg/ml. Dissolve your desired amount of antibody or protein in PBS (phosphate buffered saline), pH 7.4, e.g. 1 mg protein in PBS.

5.1.2 Add 20 µl of 10 x Component B (Coupling Buffer) to 180 µl of the 5 mg/ml protein solution and mix thoroughly by gentle vortexing or manually inverting the tube.

5.1.3 Transfer the entire volume of protein/Component B to the vial of the reactive dye (Component A) and mix thoroughly.

5.1.4 Incubate for 1 hour at room temperature with additional mixing after 30 minutes.

5.2 Purification of the Labeled Protein

5.2.1 Mount the column with a clamp on a ringstand. Remove the tip from the column and allow the buffer to run through the column into a collection tube or small beaker by gravitation.

Please note: *For your convenience the ready-to-use column does not need any equilibration. Flow will automatically stop when the meniscus reaches the disk at the top of the column packing. There is no need to worry about the column drying out since the matrix will remain hydrated.*

5.2.2 Carefully transfer the antibody-labeling mixture to the top of the column and allow the solution to enter the packing.

5.2.3 Prepare Elution Buffer by dilution of Component C, 1:10, in dest. water.

5.2.4 Add totaling 2.0 ml Elution Buffer (Component C), 1:10, in small quantities, to the top of the column.

5.2.5 Collect the faster moving band which contains the labeled protein in a clean tube as it elutes from the column. The labeled protein is dissolved in PBS, pH 7.4. and contains 2 mM sodium azide as preservative. It should be protected from excess light and stored at 2 - 6 °C.

Please note:
If desired, the remaining free dye can be removed from the column (prior to additional separations) with 12 ml of the elution buffer solution.

6. Storage and Handling of Labeled Proteins

Store the labeled protein - which will be in PBS, pH 7.4, containing sodium azide - at 2 - 6 °C, protected from light. If the final concentration of the purified protein conjugate is less than 1 mg/ml, add bovine serum albumin (BSA) or other stabilizing protein to 1 - 10 mg/ml. The conjugate should be stable at 4 °C for several months. For long-term storage, divide the solution into small aliquots and freeze at -20 °C. Avoid freeze and thaw cycles. Protect from light. Please centrifuge solutions of conjugates in a microcentrifuge before use; only the supernatant should then be used in the experiment. This step will remove any aggregates that may have formed during storage.

7. Determination of Degree of Labeling

One reaction vial (Component A) is designed to label 1 mg protein to a final molar/dye ratio between 7 and 12 (average IgG MW = 145,000).

7.1 Measure the absorbance of the labeled protein solution at 280 nm and 503 nm in a cuvette with 1 cm path length. If necessary, please dilute the protein conjugate.

7.2 Calculate the concentration of protein in the sample:

$$\text{Protein concentration (M)} = \frac{[A_{280} - (A_{503} \times 0.09)] \times \text{dilution factor}}{203.000}$$

Molar extinction coefficient of IgG: Ec = 203,000 $M^{-1}cm^{-1}$
Correction factor for absorption of MFP488 at 280 nm: CF = A_{280}/A_{max} = 0.09
Molar extinction coefficient of MFP488: Ec = 105,000 $M^{-1}cm^{-1}$

7.3 Calculate the degree of labeling:

$$\text{Moles dye per mole protein} = \frac{A_{503} \times \text{dilution factor}}{105{,}000 \times \text{protein concentration (M)}}$$

An optimal labeling of IgG's is achieved with 7 - 12 moles MFP488 dye per mole antibody.

8. Buffer Exchange/Concentration by Using Ultrafiltration Cartridges

Our ultrafiltration cartridges have a molecular weight cutoff of 10,000, i.e. purification by ultrafiltration is suited for proteins between 20 - 100 kDa.

- Buffer exchange
For optimal labeling efficiency, the purified protein must be in a buffer free of ammonium ions or primary amines. If the protein is in an unsuitable buffer, the buffer should be replaced by phosphate¬buffered saline (PBS, pH 7.4).

- Concentration of proteins
If the protein is available in a large total volume solution we recommend to concentrate the sample to smaller volumes.

Protocol

8.1 Fill ultrafiltration cartridge with your protein solution and add 200 µl 1 x Elution Buffer (PBS, pH 7.4). The maximum volume should not exceed 600 µl. Ensure lid is fully seated.

8.2 Insert assembled concentrator into centrifuge and centrifuge 10 minutes at 12,000 g. Centrifugation time is affected by sample concentration; if you obtain more than 100 µl increase centrifugation time.

8.3 Refill concentrator with 400 µl 1 x Elution Buffer and centrifuge the sample again. Repeat the process twice. The resulting protein is dissolved in PBS, pH 7.4 and contains 2 mM sodium azide as preservative. It should be stored at 2 - 6 °C.

8.4 Once the desired concentration is achieved, remove assembly and recover sample from the bottom of the concentrate pocket with a pipette.

9 Order Information, Shipping and Storage

Order#	Product	Quantity
MFP-A1235	MFP488 Protein Labeling Kit, 3 labelings	kit

Storage

Upon receipt, store Component A at -20 °C and Components B, C at 2 - 6 °C. DO NOT FREEZE THE PURIFICATION COLUMNS filled with resin. Store columns at 2 - 6 ° C. Protect Component A from light and moisture. When stored properly, the kit components should be stable for at least three months. Ultrafiltration columns should be stable for at least 1 year at room temperature.

10 Contact and Support

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